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14. ABSTRACT The long-term goal of the work proposed here is to generate, characterize and interrogate human epithelial cell-based in vivo models of prostatic carcinogenesis. These models will allow an examination of processes involved in carcinogenesis, tumor growth and metastasis. Since the tumors are themselves of human origin they represent an in vivo test bed to examine both tumor biology and the application of therapeutic agents. In the third year of funding we have completed a thorough study of metastatic spread of human prostatic epithelium from the orthotopic site using a model developed in year 2. We have generated and characterized two new normal prostatic epithelial cell lines which show promise for widespread applicability in prostate cancer research. We have continued to explore the use of lower dose Myc expressing constructs and have investigated the combination of lower levels of Myc with additional other genes commonly changed in prostate cancer to make more clinically relevant models. Specifically we have followed up on the suppression of PTEN described in the second report and have also combined suppression of PPAR. with expression of cMyc to produce enhancement of a mild myc-induced phenotype.

15. SUBJECT TERMS
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Introduction

The **long-term goal** of the work proposed here is to generate, characterize and interrogate human epithelial cell-based in vivo models of prostatic carcinogenesis. These models will allow an examination of processes involved in carcinogenesis, tumor growth and metastasis. Since the tumors are themselves of human origin they represent an in vivo testbed to examine both tumor biology and the application of therapeutic agents.

As proposed we have generated and used models in which human prostatic epithelial cells (huPrE) are grown in tissue recombinants with rat urogenital sinus mesenchyme (rUGM) and grafted back into the in vivo environment of an intact male athymic rat host. Manipulations of the huPrE allow us to examine the effects of retroviral transfection with c-Myc within the huPrE. Our original C7-Myc model forms aggressive tumors which move rapidly from a benign to metastatic phenotype. Hence, we have generated new molecular and cellular tools and from these have made less aggressive models (as originally proposed), which allow us to follow the progressive events in cancer initiation and progression. In combination with this approach we have started to modify specific signaling pathways which are known to be altered in human prostate cancer to examine their effects in combination with those of c-Myc.

Work Ongoing and Completed

In the present funding period we have completed analysis of the orthotopic grafting model which was described in the previous annual report. Briefly we have developed a new technique for grafting human prostate cancer cells within the lumen of murine prostatic ducts. This allows tumors to form in a manner analogous to that seen in human patients. Of particular significance, grafts to this site metastasize in a pattern similar to that seen in human patients. The tumor cells migrate along the spinal column and invade the spine and major bones as well as the liver, lungs and other organs. This is important because metastatic spread to the bone is an important biological component of human prostate cancer which has not been easy to model in the in vivo systems used historically. Dr. Jiang, who developed this model and is currently writing a descriptive paper, will follow-up on this aspect of the work by submitting a New Investigator proposal which will explore potential medical approaches to inhibit metastasis to bone using this technique.

As described in the first two annual reports the original C7-Myc was extremely aggressive and therefore of limited practical use. We have therefore developed new human prostatic epithelial cell lines (NHPrE

and BHPrE) which are able to replicate many critical aspects of human prostate including expression of both androgen receptors and PSA (figure 1).

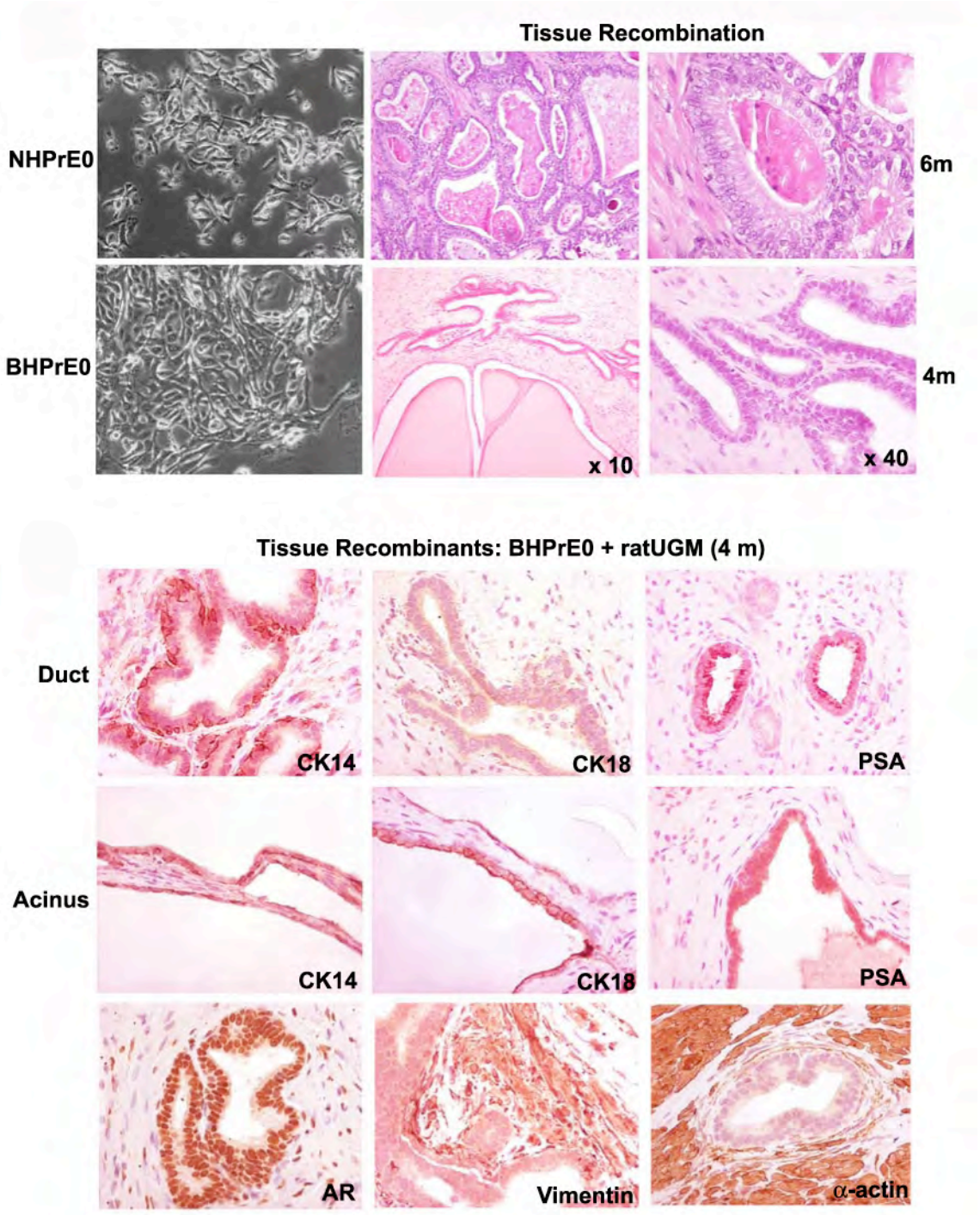

Figure 1. NHPrE and BHPrE cells recombined with rat urogenital sinus mesenchyme. The two cell lines both form glandular structures which show appropriate stromal and epithelial organization and marker expression.

In order to investigate whether overexpression of c-Myc resulted in carcinogenesis in these cells we introduced the C7-Myc construct and generated tissue recombinants using rUGM.

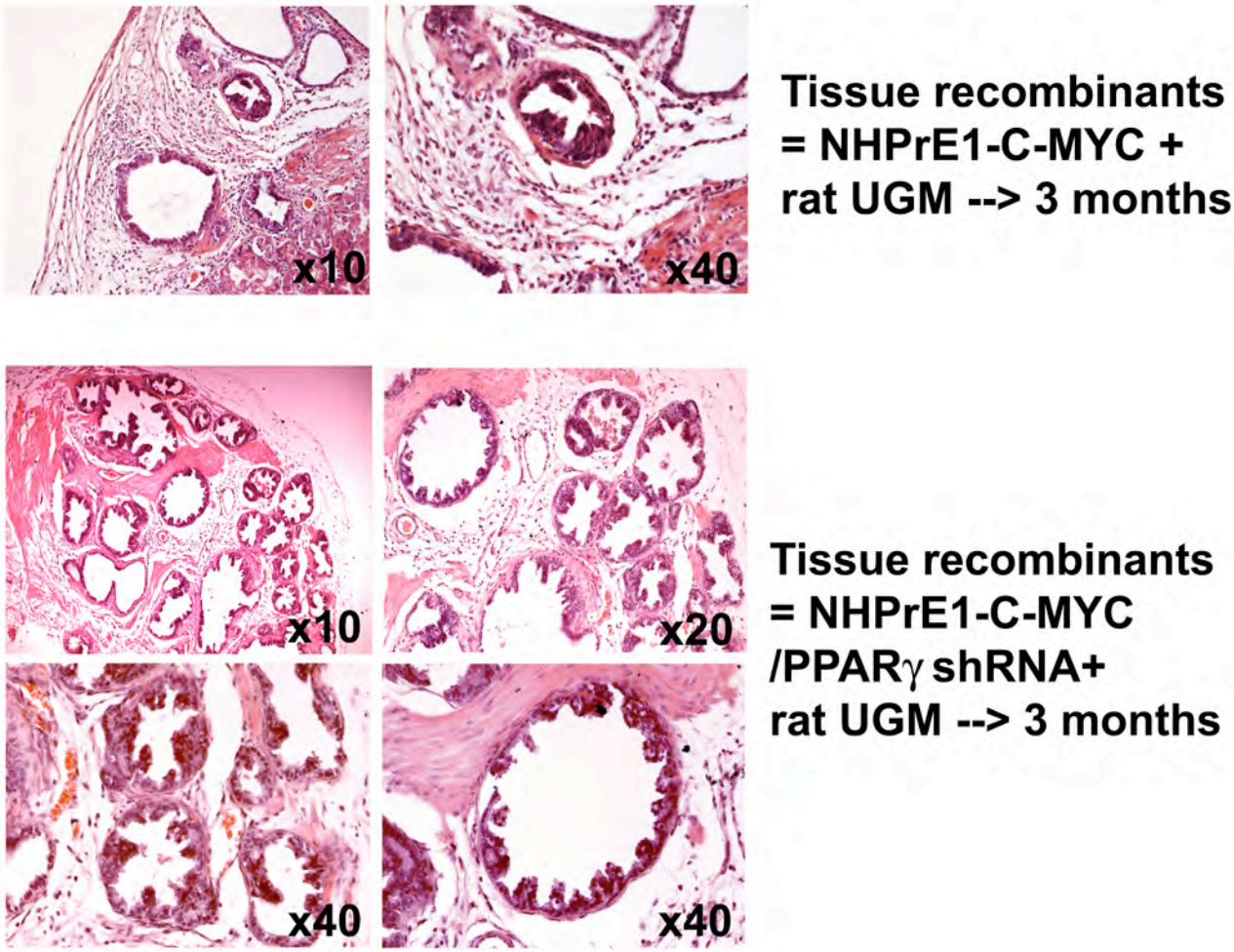

Figure 2. Consequences of c-Myc overexpression and PPARγ suppression on the differentiaiotn of NHPrE +rUGM recombinants. In the upper panel it is evident that overexpression of c-Myc in the epithelial cells resulted in the formation of preneoplastic PIN lesions. In the lower panel additional suppression of PPARγ enhanced the frequency and severity of this effect.

When c-Myc was overexpressed in the NHPrE cells no evidence of malignant progression was seen over a three month experimental period (figure 2). In accordance with the concepts outlined in specific aim 3 we therefore added additional genetic insults to the model. In the lower panel of figure 2 the suppression of PPARγ signaling in the c-Myc overexpressing epithelial cells resulted in a more severe and widespread PIN phenotype than overexpression of c-Myc alone. Loss of PPARγ signaling due to loss of enzymes making the ligands for this nuclear receptor is a common occurrence in early human prostate cancer.

In the previous report we showed that loss of PTEN expression in human prostatic epithelial cells was sufficient to elicit a PIN phenotype. We have therefore added this additional third insult to the cells in which c-Myc is overexpressed and PPARγ suppressed in an attempt to generate a more aggressive and invasive model. Results for this experiment are pending at this writing.

In a further attempt to make this third insult more subject to intentional manipulation we have generated a tetracycline regulatable version of the PTEN suppression shRNA construct (figure 3). As shown in the figure 3 this construct responds to tetracycline by suppressing PTEN expression with a concomitant rise in Akt phosphorylation. This can be utilized to further fine tune the progression model as necessary.

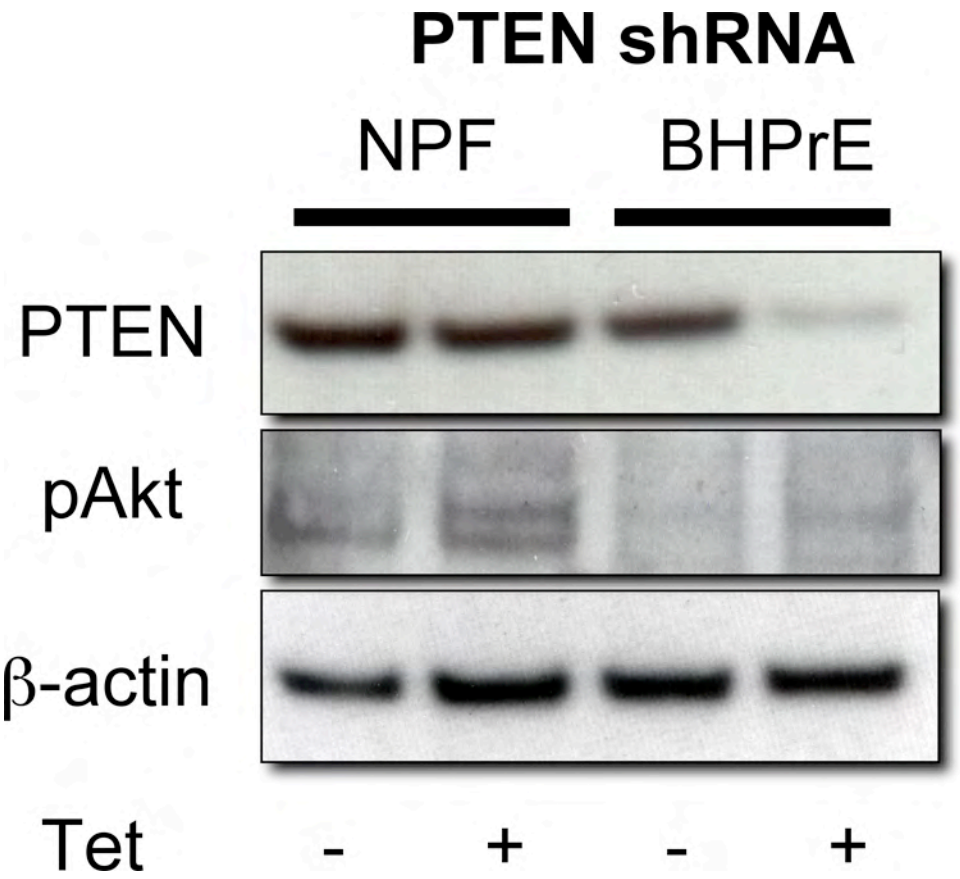

Figure 3. Regulation of PTEN expression in normal human prostate fibroblasts (NPF) and in the BHPrE epithelial cell line using a virally transduced tetracycline regulated shRNA construct.

In additional related experiments we have been able to show, in a collaboration with the Bhowmick laboratory, that expression of c-Myc in prostatic stromal cells results in phenotypic changes consistent

with those seen in human prostatic carcinoma associated fibroblasts (CAF). This adds to data, some published and some not, showing that the CAF phenotype is complex and can result from a number of different genetic and phenotypic changes.

In an effort to pursue data on the role of gene expression in the genesis of prostate cancer we have also been (somewhat peripherally) involved in a project with the Matusik laboratory which has resulted in the generation of a new mouse model of prostate cancer based upon the downregulation of the cell cycle control regulator p57Kip2 which results in a prostatic phenotype which closely resembles human prostatic carcinogenesis (paper in press).

Technical Modifications

As described in the previous report we have modified the orthotopic site graft method to use intraductal grafting which has proven to give a more reliable pattern of metastatic spread, more closely resembling that seen in human prostate cancer patients. We have also included the use of a number of human prostatic epithelial cell types which were not available at the time this proposal was written. These allow more consistent data to be generated than the proposed use of primary epithelial cultures. Further in the present period we have generated new models which show a more measured development of malignancy than that seen in previous models and have developed tools to selectively modify this progression using regulatable suppression of PTEN.

Personnel Changes

None since the last report

Key Research Accomplishments

(Previous periods)

- *Development of a new model of prostate cancer metastasis based upon orthotopic intraductal xenografting.*
- *Development and characterization of tet-regulated c-Myc models using human prostatic epithelium.*
- *Characterization of viral vectors to suppress PTEN expression and to activate Akt signaling in tissue recombinants using human prostatic epithelium and rUGM.*

(Current Period)

- Generation and in vivo testing of two new benign human prostatic cell lines which recapitulate normal prostatic developmental milestones including expression of androgen receptor and PSA and which can serve as a basis for further model development based upon specific genetic changes.
- Establishment of new cells lines (based on the normal lines described above) overexpressing c-Myc both alone and in combination with suppression of PPARγ. These models provide a much more measured progression to malignancy than the original C7-Myc line. This work also demonstrates that two common early changes in human prostate cancer can act additively to induce a phenotypic response.
- Development of tet-regulated PTEN suppression models.
- One paper is in press, one submitted and a further two are in preparation in the current period.

Reportable Outcomes.

Jin, R.J., Lho, Y., Wang, Y., Ao, M., Revelo, M.P., Hayward, S.W., Wills, M.L., Logan, S.K., Zhang, P. and Matusik, R.J. [2008] Down regulation of p57Kip2 induces prostate cancer in the mouse Cancer Research (in press)

Conclusions.

So far this project has generated a number of important new model systems and techniques. Notable amongst these are the development of a new orthotopic metastasis model. This will be useful for many future studies. We have also generated a number of new cell lines which will expand the repertoire available to the research community. These have been used as a basis for new models and have shown their ability to act as specific

We have requested a no cost extension of this project in order to complete a few final experiments and more pressingly to complete a number of outstanding manuscripts which have been supported by this funding. This will allow descriptions of the models and reagents generated to the wider scientific community and for their subsequent distribution to interested parties. Currently one manuscript is in press (see above) one has been submitted (a collaboration with the Tang laboratory at M.D. Anderson Cancer Center), two papers are in late stages of preparation, one describing the orthotopic modeling technology and the other describing the new benign cell lines. In addition work is being completed to allow a full description of the combinations of c-Myc expression alone, with PPARγ suppression and

with the addition of PTEN suppression. This work provides a series of novel and biologically relevant in vivo models with which to continue exploring the role of genetic changes of human prostatic epithelium in prostate cancer.